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Innate immunity to intracellular pathogens: macrophage receptors and responses to microbial entry

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Summary: Innate immunity to intracellular pathogens encompasses a range of interactions of cellular and humoral activities of the host with the invading microorganism, determining the outcome of infection. Here, we review the particular role of macrophage recognition receptors and effector responses in the uptake of microbes and their products. We place this in context and raise issues for discussion and further experimentation.

Keywords: *innate immunity, macrophages, receptors, intracellular pathogens, phagocytosis*

Introduction

It is widely held that interactions between the host and microorganism have played a critical role in the evolution of the immune system, imposing reciprocal selective pressures and diverse invasion and evasion strategies on both participants. Mutual adaptation is necessary to ensure genome survival and replication of both partners, the final measures of fitness and natural selection. While coexistence and symbiosis are the ultimate signs of successful parasitism, pathogenic interactions destabilize and disrupt the host-protective mechanisms of innate and adaptive immunity. The ability to enter and survive within host cells is essential for obligate and facultative microbes, but organisms able to replicate extracellularly cannot avoid uptake of intact or degraded components by phagocytic cells and triggering of an innate immune response. Microbial strategies to survive killing mechanisms have taught us a great deal of cell biology and provide targets for treatment of many serious chronic infections, which constitute a large burden of morbidity and mortality in human and veterinary disease.

The innate immune system, which has achieved extraordinary success in antimicrobial resistance to infection, encompasses a range of specialized hemopoietic cell types such as phagocytes [neutrophils, monocytes, macrophages,

and myeloid dendritic cells (DCs)] and innate lymphocytes [natural killer (NK) cells, NKT cells, γ/δ T cells, and plasmacytoid DCs], in addition to mast cells, basophils, eosinophils, and newly described nuocytes (1). Even platelets and erythrocytes contribute to host resistance; endothelia, epithelia, and connective tissue are important interfaces and biosynthetically active barriers to the spread of infection. In a sense, all metabolically active cells including hepatocytes and neuro-endocrine cells are directly or indirectly implicated in systemic homeostasis and inflammation. In this review, we focus on innate mechanisms of the response of 'professional' phagocytes, especially macrophages, to a broad range of microorganisms, to illustrate the diverse mechanisms of resistance, initiation of adaptive immunity and pathologic consequences of infection. We consider not only microorganisms traditionally regarded as intracellular pathogens but also others that can infect and replicate in macrophages, such as *Neisseria meningitidis* and *Candida albicans*, as innate recognition and responses have many features in common.

History

In parallel with the heroic age of microbiology, study of humoral mechanisms of immunity yielded chemical knowledge of antibodies and complement. Elie Metchnikoff is justly regarded as the founder of cellular innate immunity, especially phagocytosis, microbial killing, and digestion, as integral mediators of inflammation and host defense (2). From the outset, the study of mycobacterial infection by Robert Koch, Elie Metchnikoff, and later Florence Sabin, for example, provided a leitmotiv in immunologic research, culminating in the modern cell biological exploration of a range of infectious models of disease. These include pioneering studies by James Hirsch and Thomas Jones (*Toxoplasma*), George Mackaness (*Listeria*, BCG), Philip D'Arcy Hart (*Mycobacterium tuberculosis*), Samuel Silverstein and Marcus Horwitz (*Legionella*), and Michel Rabinovitch (*Leishmania*, *Coxiella*), among others. Cedric Mims, MacFarlane Burnet, and Alick Isaacs stimulated early interest in virus interactions with mammalian cells, with the discovery of interferon as a remarkable antiviral product of infection. Advances by George Palade, Christian de Duve, Zanvil Cohn, and others brought insights into cellular secretion pathways, endocytosis, and digestion. Studies of phagocytosis of organisms including bacteria, yeast particles (zymosan), and protozoans, provided fresh insights into host–pathogen interactions, providing a basis for later explosive progress in molecular biology. The discovery of Toll-like receptors (TLRs) and the concept of germline-encoded recognition receptors dovetailed with longstanding interest in lipopolysaccharide (LPS) to spur interest in innate immunity. The modern synthesis of genetics of host and pathogen utilizes model organisms (*Drosophila*, *Caenorhabditis elegans*, zebrafish) to study infection in mice and humans. ENU mutagenesis and systems biology now go hand in hand with microbiology and immunology to explore recognition, cell responses, and antimicrobial mechanisms *in vivo* and *in vitro*.

Innate host defense to infection

Humoral immunity, especially complement activation, shares features and co-operation with innate cellular recognition and effector mechanisms. Innate lymphocytes such as NK and NKT cells, display specialized mechanisms of recognition and response. The basic biology of production, distribution, recruitment, and defense mechanisms of neutrophils, monocytes/macrophages, and DCs is well documented. Here, we deal mainly with principles and topics relating to macrophage–pathogen interactions. For overviews of these topics see previously published reviews (3–5).

Monocyte and macrophage heterogeneity

Contemporary studies have stimulated interest in the growth and differentiation of mononuclear phagocytes, especially the description of fetal liver, bone marrow, and blood monocyte-derived subsets that give rise to resident tissue macrophages, present in all organs in the absence of inflammation, and newly recruited monocytes that give rise to inflammatory and regulatory macrophages in tissues at sites of infection (6, 7). It is convenient to characterize the stereotypic phenotype of such macrophages by antigenic [immunocytochemistry, fluorescence-assisted cell sorting (FACS)], microarray, and proteomic analyses. The response of macrophages to different activatory and inhibitory ligands results in complex modulation in phenotype, with considerable heterogeneity, and still poorly defined mechanisms of their plasticity. We have classified the activation pathways of these macrophages as innate (directly activated by microbial stimuli, often via TLRs), classically activated [induced by interferon-γ, also known as M1 macrophages, by analogy with T-helper 1 (Th1) lymphocytes], and alternatively activated by the Th2 cytokines, interleukin-4 (IL-4) and IL-13 (M2 macrophages) (8). Deactivation by cytokines such as IL-10 and transforming growth factor-β (TGF-β), by glucocorticoids and prostanoids, or by plasma membrane or intracellular inhibitors counterbalance and limit macrophage hyper-reactivity. The interaction with diverse microorganisms (bacteria, viruses, fungi,

parasites) is profoundly influenced by the differentiation and activation status of individual macrophages and heterogeneous populations in different compartments of the body.

Neutrophils share many of these functions, while mainly representing short-lived, newly recruited cells at infected tissue sites. They are vital to survival of the host to acute bacterial challenge, and display potent antibacterial and antifungal activities. They store preformed antimicrobial and digestive molecules for rapid release by degranulation, yet are more biosynthetically active than initially appreciated. They share a respiratory burst with activated macrophages and are the source of much of our knowledge of its components and dysfunction. Although neutrophils have been implicated in the capture, survival, and transmission of intracellular pathogens such as *Leishmania*, they provide a considerably more hostile intracellular environment than other phagocytes to infection (9).

Myeloid DCs also share many features with macrophages but are specialized to capture, process, and present microbial antigens to naive T lymphocytes, providing a bridge to adaptive, antigen-specific effector and memory responses (10). The plasmacytoid DCs are poorly or non-phagocytic producers of Type-I interferons, which in turn have complex effects on the functions of macrophages and other cells in innate immunity (11).

Antigen-presenting cell (macrophage/DC) interactions with microorganisms: general aspects

Recognition and sensing

Elsewhere in this volume, the focus is on individual intracellular microbes. We attempt here to compare the macrophage responses to a range of microorganisms, to bring out common as well as unique features of the macrophage–pathogen relationship. Mononuclear phagocytes express a range of receptors involved in the recognition of diverse microbial ligands (12, 13). Examples of receptors are illustrated in *Fig. 1*, and known microbial ligands are listed in *Table 1*. Receptors include opsonic (activatory and inhibitory FcRs, complement receptors) and non-opsonic receptors (lectins such as Dectin 1 and DC SIGN/SIGN R1, and scavenger receptors such as SR-A I/II and MARCO). Sensing is mainly an attribute of the TLRs, at the plasma membrane or within the endocytic vacuolar compartment. Cytosolic recognition depends on protein assemblies such as the nucleotide oligomerization domain (NOD)-like receptor (NLR) and RIG-I-helicase receptor (RLR) families of sensors. Expression of these receptors varies on monocytes, macrophages, and DCs, depending on the species, tissue microenvironment and activation. Receptors involved in the recognition and uptake of intact bacteria and fungi, for example, collaborate with one another as multiprotein complexes, contributing to high affinity binding and signal transduction. Examples of the role of some of these receptors in microbial recognition and entry are illustrated in the following subsection.

Macrophage receptors and microbial recognition

Several macrophage receptors have been shown to be involved in microbial recognition and uptake. One example is the class A scavenger receptor (SR-A). SR-A is a phagocytic receptor, mediating non-opsonic phagocytosis of several bacterial pathogens, including *Neisseria meningitidis* (14, 15). Bacterial ligands for SR-A include LPS from Gram-negative bacteria, lipoteichoic acid from Gram-positive bacteria (16), bacterial CpG DNA (17), as well as bacterial surface proteins (18). The role of SR-A in the uptake of *N. meningitidis* has been shown *in vivo*, where survival and health scores of SR-A knockout ($SR\text{-}A^{-/-}$) mice were significantly lower than for wildtype mice (18). This study confirmed the role of meningococcal surface proteins in SR-A-mediated uptake. $SR\text{-}A^{-/-}$ mice have also been shown to be more susceptible to experimental *Listeria monocytogenes* and *Staphylococcus aureus* infection as a result of deficient bacterial clearance from the spleen and liver (19, 20). In essence, SR-A protects mice against systemic tumor necrosis factor (TNF) release and septic shock.

Streptococcus pyogenes and the group B streptococcus (GBS; *Streptococcus agalactiae*) have evolved mechanisms to evade recognition by macrophages via SR-A. In both strains the virulence factors (the *S. pyogenes* M protein and the GBS polysaccharide capsule) prevent recognition and non-opsonic phagocytosis of streptococci by SR-A on macrophages, most likely by masking the SR-A ligand(s) at the bacterial surface (21). Another class A scavenger receptor, MARCO (macrophage receptor with collagenous structure) has been shown to be involved in host defense against *S. pneumoniae* (22) and *N. meningitidis* (23). Downregulation of MARCO on alveolar macrophages has also been linked to possible enhanced susceptibility to secondary pneumococcal infection subsequent to an influenza infection (24). The mannose receptor (MR) has been implicated in Dengue virus infection of macrophages, and it is markedly enhanced by IL-4/13, inducers of alternatively activated macrophages. Other lectins such as DC-SIGN/SignR1 enhance uptake of Dengue virus and human immunodeficiency virus-1 by APCs (25).

Table 1. **Pattern recognition receptors and microbial ligand recognition**

Pattern recognition receptor	Localization	Ligand	Ligand origin
Scavenger receptors			
SR-A	Plasma membrane	LPS, LTA, CpG DNA, proteins	Bacteria
MARCO	Plasma membrane	LPS, proteins	Bacteria
CD36	Plasma membrane	Diacylated lipopeptide	Bacteria
LOX-1	Plasma membrane	Protein	Bacteria
SREC	Plasma membrane	Protein	Bacteria
C-type lectins			
DC-SIGN	Plasma membrane	LPS, ManLAM, CPS, CTL	Bacteria Virus Protozoa
Mannose receptor	Plasma membrane	LPS, CPS, ManLAM	Bacteria Virus Fungi Protozoa
Dectin-1	Plasma membrane	β-Glucan, mycobacterial ligand	Fungi
Dectin-2	Plasma membrane	β-Glucan, high mannose structures	Fungi
MINCLE	Plasma membrane	SAP130	Fungi
Toll-like receptors			
TLR1	Plasma membrane	Triacyl lipoprotein	Bacteria
TLR2	Plasma membrane	PGN, porins, lipoarabinomannan HA protein tGPI-mucin	Bacteria Viruses Protozoa
TLR3	Endolysosome	dsRNA	Virus
TLR4	Plasma membrane	LPS Envelope proteins	Bacteria Viruses
TLR5	Plasma membrane	Flagellin	Bacteria
TLR6	Plasma membrane	Diacyl lipoprotein	Bacteria Viruses
TLR7 (human TLR8)	Endolysosome	ssRNA	Bacteria Viruses
TLR9	Endolysosome	CpG DNA DNA Malaria hemozoin	Bacteria Viruses Protozoa
TLR10	Endolysosome	Unknown	Unknown
TLR11	Plasma membrane	Profilin-like molecule	Protozoa
NOD-like receptors			
NOD1	Cytoplasm	iE-DAP	Bacteria
NOD2	Cytoplasm	MDP	Bacteria
NLRP1	Cytoplasm	MDP, Anthrax lethal toxin	Bacteria
NLRP3	Cytoplasm	RNA, LPS, LTA, MDP Viral RNA	Bacteria Viruses Protozoa Fungi
NLRC4	Cytoplasm	Flagellin	Bacteria
Naip5	Cytoplasm	Flagellin	Bacteria
RIG-like receptors			
RIG-I	Cytoplasm	Short dsRNA, 5′ triphosphate dsRNA	RNA viruses DNA viruses
MDA5	Cytoplasm	Long dsRNA	RNA viruses
LGP2	Cytoplasm	RNA	RNA viruses
Other receptors			
CD14	Plasma membrane	Peptidoglycan, LTA, LPS, mannuronic acid	Bacteria
CR3	Plasma membrane	Oligosaccharides, microbial protein β-Glucans	Bacteria Fungi
TREM2/DAP12	Plasma membrane	LPS, microbial molecules	Bacteria Fungi
TREM1/DAP12	Plasma membrane	Unknown	Bacteria
Clec5(MDL-1)/DAP12	Plasma membrane	Unknown	Virus

CPS, capsular polysaccharide; CTL, cytoxic T lymphocytes; LPS, lipopolysaccharide; LTA, lipteichoic acid; MARCO, macrophage receptor with collagenous structure; MDP, muramyl dipeptide; NOD, nucleotide oligomerization domain; SREC, scavenger receptor expressed by endothelial cell-I.
References: (12, 56, 97–100).

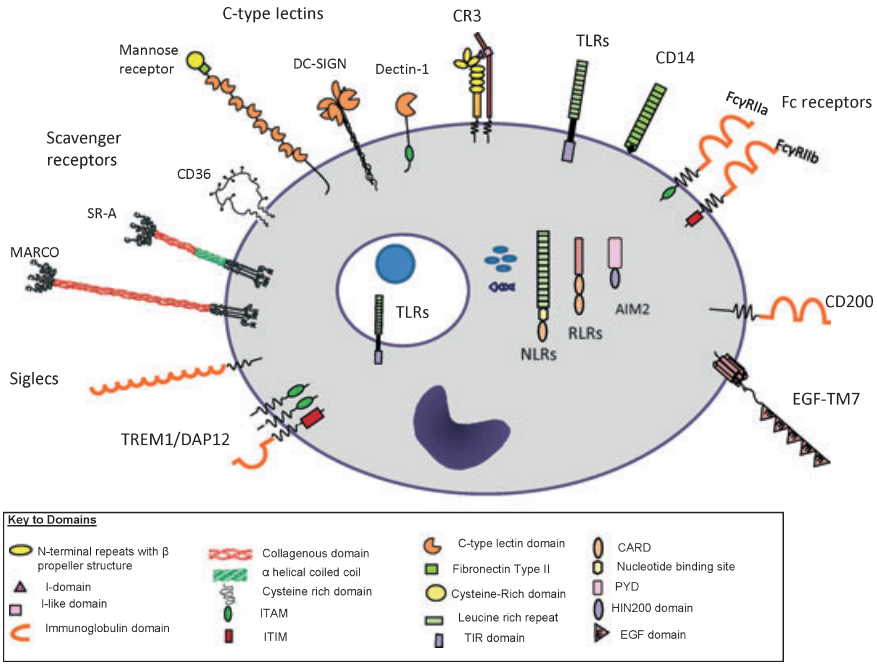

***Fig. 1.* Schematic structures of selected macrophage receptors implicated in microbial recognition.** Macrophages express a wide variety of receptors which mediate recognition of microbial pathogens. Phagocytic surface receptors include non-opsonic receptors (e.g. C-type lectins and Scavenger Receptors) as well as opsonic receptors (e.g. complement receptor and Fc receptors). Toll-like receptors (TLRs) are sensing receptors, some of which are expressed on the surface (e.g. TLR4), while others are vacuolar (e.g. TLR9). Sensing receptors are also found in the cytoplasm, and these include the NOD-like receptors (NLRs), RIG-like receptors (RLRs), and DNA sensors (e.g. AIM2). Other surface molecules regulate the response of myeloid cells to unrelated agonists, for example the EGF-TM7 molecule EMR2, as well as TREM1/2 and CD200/CD200R. AIM2, absent in melanoma 2; CARD, caspase recruitment and activation domain; CR3, complement receptor 3; DAP12, DNAX activation protein of 12 kDa; EGF-TM7, epidermal growth factor seven transmembrane receptor; FcγR, Fc gamma receptor; HIN200, Hin 200 domain; family; ITAM/ITIM; immunoreceptor tyrosine-based activation/inhibition motif; MARCO, macrophage receptor with collagenous structure; NLRs, NOD-like receptors; PYD, pyrin domain; RLRs, RIG-like receptors; SR-A, scavenger receptor A; Siglecs, sialic acid binding Ig-like lectins; TLRs, Toll-like receptors; TREM1, triggering receptor expressed on myeloid cells-1.

An example of a macrophage receptor involved in the innate immune recognition of fungal pathogens is Dectin-1, a C-type lectin receptor which recognizes β-glucan (26). The receptor possesses a cytoplasmic immunoreceptor tyrosine-based activation motif-like sequence that is involved in mediating proinflammatory cytokine production in response to β-glucan, in cooperation with TLR2 (27, 28). Dectin-1 can also stimulate the oxidative burst in response to β-glucans, independently of the TLR pathway. It has been shown to play an important role in the uptake of *Candida albicans* and studies have shown uptake is dependent on the exposure of glucan by the yeast form but not the filamentous form. This may provide a basis for fungal evasion mechanisms (29). Another study showed that the MR, which accumulates on phagosomes at later stages, has a potential role in phagosome sampling, subsequent to Dectin-1 (30).

The role of the above innate recognition, non-opsonic receptors in the uptake of a variety of intracellular bacteria has not been studied, except in the case of mycobacterial infection. SR-A was redundant for infection in mice (31), whereas MARCO plays a role in the pro-inflammatory response to trehalose dimycolate (47), as does the lectin-like receptor Mincle (32). CD36, an unrelated scavenger receptor, has also been implicated in mycobacterial stimulation of TNF release by macrophages (33).

Uptake

Depending on its size and bulk, uptake of a microbial particle is usually by phagocytosis, or modifications thereof, induced by selected pathogens (4) (*Fig.* 2). Viruses can enter via endocytosis, macropinocytosis, or fusion. Complex signaling pathways, cytoskeleton remodeling, dynamic membrane movements, fission and vesicular fusion, and relays of phosphorylation/dephosphorylation are set in train. Intravacuolar processes such as acidification and killing and digestion determine the outcome of infection. Cytoplasmic proteins enter the nucleus and these transcription factors regulate gene expression, biosynthesis, and secretion of pro- and anti-inflammatory molecules (proteins such as cytokines, chemokines,

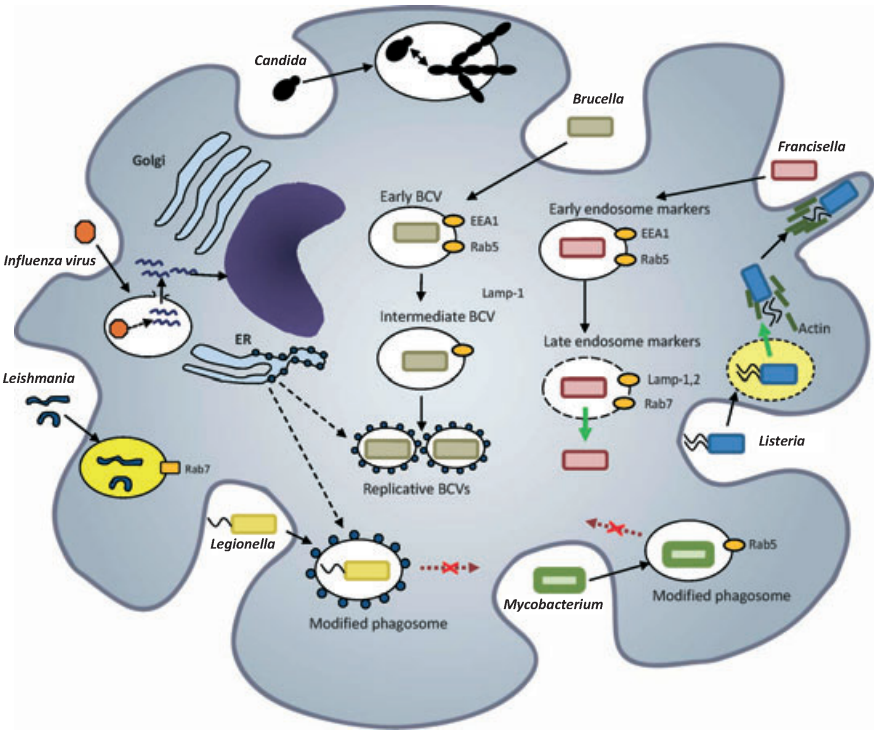

Fig. 2. Selected pathogens evade distinct phagocytic mechanisms. Pathogens have developed several mechanisms to enter and survive inside macrophages. The dimorphic fungus *Candida albicans* undergoes a conversion from a unicellular form to a multicellular hyphal form, which allows this fungus to escape from the macrophage. Uptake of the yeast is characterized by localized actin polymerization, phosphoinositide accumulation, Syk kinase activation, and rapid acquisition of phagolysosomal markers. Following an LPS-dependent, lipid raft-mediated entry, *Brucella abortus* is found in an early *Brucella*-containing vacuole (BCV) that acquires early endosome markers Rab5 and EEA-1. BCVs then mature into acidic intermediate vacuoles that accumulate LAMP-1, but not Rab7, avoiding interactions with late endosomes and fusion with lysosomes. BCVs interact with ER exit sites (via VirB type IV secretion system) leading to fusion with the ER, generating an ER-derived organelle permissive for bacterial replication. Replicative BCVs exclude LAMP-1 and acquire various ER markers as a result of membrane exchange with the ER. Bacterial replication is thought to occur through fission of the BCV into two daughter BCVs via further accretion of ER membranes. The *Francisella tularensis* phagosome acquires the early endosome markers EEA1 and Rab5 and then matures into a late endosome defined by the presence of the markers Lamp1, Lamp2, and Rab7. The late endosome does not acidify, and the phagosomal membrane is disrupted, releasing the bacteria into the cytosol. Acidification of the *Listeria monocytogenes* phagosome is essential for the perforation of the phagosomal membrane and escape of the bacteria into the cytosol. Here, they mobilize the actin polymerization machinery to move within the cell and then from cell to cell. The *Mycobacterium tuberculosis* phagosome acquires the early endosome marker Rab5 but excludes the late endosomal Lamps and Rab7. This organism also produces molecules that block fusion with the lysosome and resides and replicates in this early endosome. *Legionella pneumophila* resides and multiplies in a vacuole studded with ribosomes due to interaction with the rough endoplasmic reticulum (RER). The organism secretes effector molecules via its type IV secretion system into the cell which inhibit phagosome/lysosome fusion. Another example not shown is *Yersinia*, which transports several virulence factors into the host cytosol via a type three secretion system. These proteins function to counteract multiple signaling responses in the infected host, including responses initiated by phagocytic and Toll-like receptors, thereby disrupting the innate and adaptive immune responses. The *Leishmania mexicana* phagosome develops into an acidic phagolysosome containing Rab7 where the parasite is able to survive and replicate. Viruses such as the influenza virus are able to inhibit the activation of antiviral mechanisms, such as the activation of interferon regulatory function (IRF) proteins which induce interferon production upon viral infection, and enter the nucleus. Cytomegalovirus (not shown) incapacitates a range of MHC-antigen presenting pathways (4, 91–93). Adapted from (94).

proteolytic enzymes) and low molecular weight metabolites (reactive oxygen and nitrogen, arachidonates). Host cellular responses include inflammasome activation, autophagy, apoptosis, and necrosis. Protective host reactions to infection include negative regulation of TLRs, NLRs, and inflammasome activation by inhibitory surface molecules such as CD200/CD200R (34) and suppression of cytokine synthesis (SOCS) (35) inhibitory proteins. Induction of Heme oxygenase 1 (36), heat shock proteins (HSPs) (37), and of stress-induced chaperones and antioxidants limit cell and tissue injury. Environmental factors such as hypoxia (38), fever, and iron availability (39) influence the outcome of host–pathogen interactions, in part through modulation of transporter proteins such as Nramp, a genetically polymorphic molecule implicated in several intracellular infections of macrophages (*Leishmania*, *Mycobacteria*, *Salmonella*) (40).

Fig. 2 illustrates schematically some of the diverse cellular pathways employed by selected intracellular pathogens during macrophage infection (41). Each step in the infection process can be exploited by pathogens, including uptake via a novel

phagosome (*Legionella*) (42), inhibition of phagosome–endosome fusion and acidification (*Toxoplasma*, *Mycobacteria*) (43, 44). Organisms such as *Leishmania* are able to replicate in phagolysosomes, others such as *Listeria monocytogenes* lyse the phagosome membrane and escape into the cytosol, where it initiates actin assembly, and propulsion within and between cells. Viruses can enter by fusion from an endosomal compartment. Bacteria such as *Brucella* colonize the endoplasmic reticulum and Golgi compartment (45), whereas *Legionella* and *Toxoplasma* attract RER and mitochondria for ill-defined functions. Opsonization by antibody and/or complement diverts the incoming pathogen to a conventional phagolysosomal compartment, followed by its destruction (46).

Induction of macrophage homokaryon formation is a prominent feature of *M. tuberculosis* infection. The mechanism of fusion and its potential selective advantage to the host or pathogen remain unclear. In the case of schistosomiasis, a strong inducer of alternative activation of macrophages, fusion results from the action of Th2 cytokines, IL-4/13, and signal transducer and activator of transcription 6 (Stat6) (8).

Killing mechanisms

After internalization of cargo, phagosomes subsequently fuse with intracellular granules to form the phagolysosome, within which microbial killing is achieved by a combination of non-oxidative and oxidative mechanisms (47–50). Potent non-oxidative killing mechanisms include antimicrobial peptides (AMPs) such as cathelicidins and defensins and the activities of cathepsins and other degradative proteases; the oxygen-dependent 'respiratory burst' involves the non-mitochondrial generation of antimicrobial reactive oxygen species (ROS) through the membrane-bound NADPH oxidase enzyme complex. Inducible nitric oxide synthase (iNOS), expressed in the plasma membrane after activation of macrophages by interferon-γ, constitutes a second major defense mechanism of classically activated macrophages. These different mediators interact with and potentiate antimicrobial activity. For example, macrophage expression of the murine cathelicidin-related antimicrobial peptide (CRAMP) is increased after infection by the intracellular pathogen *Salmonella typhimurium*, and this increase is dependent on intracellular reactive oxygen intermediates (51). Conversely, microorganisms have evolved a host of defenses against oxidative killing (52). In the mouse, immunity-related GTPases (IRGs), induced by interferon-γ, accumulate at the parasitophorous vacuole of invading *Toxoplasma gondii*, for example, leading to the destruction of vacuole and parasite. Genetic deficiency of Irgm1 or the autophagic regulator Atg5 leads to spontaneous activation of IRG proteins and cell cytotoxicity (53).

Some microbial breakdown products enter the cytosol by poorly defined mechanisms and are recognized by intracellular receptors/sensors (NLRs, RLRs, and DNA sensors such as AIM2) (54–57). Recognition of microbial molecules by these receptors mediates inflammasome activation, caspase cleavage, and the release of IL-1 (58) (*Fig.* 3).

Apart from phagocytosis and degranulation, neutrophils and probably activated macrophages also employ the formation of neutrophil extracellular traps (NETs), which arise from the release of neutrophil nuclear contents into the extracellular space (9, 59). NETs are composed of decondensed chromatin decorated with granular and cytoplasmic antimicrobial proteins (e.g. myeloperoxidase, serine proteinases, histones) (60). They bind and kill a variety of microbes including bacteria, fungi, and parasites and may play a role in the killing of pathogens that are too large to be phagocytosed (e.g. helminths).

Antigen processing and presentation

The details of major histocompatibility complex (MHC)-dependent antigen processing and presentation by myeloid DCs are described elsewhere (61). Here, we note the ability of pathogens to promote or subvert this process, for example by modulating the maturation of DCs, inducing expression of costimulatory molecules, or by targeting biosynthesis or stability of key components involved in the activation of naive T lymphocytes. The suppressive effects of chronic intracellular pathogens on antigen-presenting cell (APC) functions are largely unknown.

Macrophage modulation by infection

Recent studies have provided insights into the complex dynamic interactions between specific pathogens and macrophages. *Fig.* 4 illustrates the remarkable ability of microbes to modulate the phenotype of the macrophage, inducing the cell to switch from a pro-inflammatory (M1) to an alternatively activated (M2) phenotype. *Francisella tularensis* (FT) ensures its survival at the expense of the macrophage, whereas respiratory syncytial virus (RSV) promotes repair of the host at sites of infection (62, 63).

Issues regarding the role of innate immunity in the response to intracellular infection

After the above overview of the innate responses of phagocytes to intracellular microbes and their products, we raise

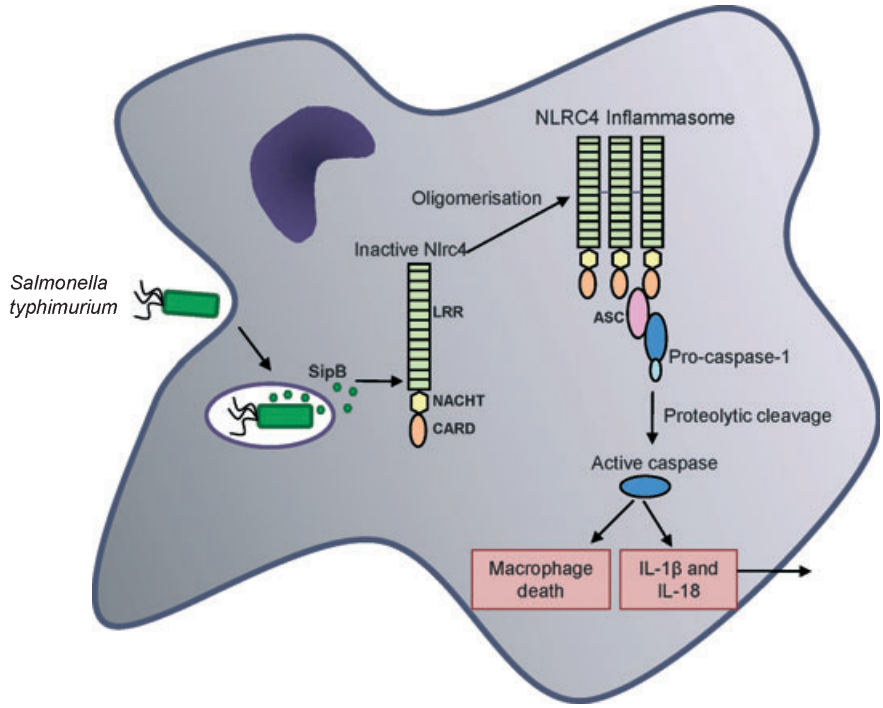

Fig. 3. Macrophage sensing of the intracellular pathogen *Salmonella typhimurium* is mediated by the NLRC4 inflammasome, which detects monomeric flagellin secreted by the bacterial type III secretion system and is dependent on the *S. typhimurium* SipB protein. Inactive monomeric Nlrc4 (formerly IPAF, ICE-protease activating factor) oligomerises to form active NLRC4 inflammasome, recruiting ASC (apoptosis-associated speck-like protein containing a CARD system) which, in turn, recruits procaspase-1. Proteolytic cleavage activates caspase-1, inducing the release of interleukin-1β, interleukin-18, and macrophage cell death (94).

issues for discussion and further investigation. These relate to the specificity of discrimination and the diversity of responses, depending on the nature of the particular organism, type of immunity, and the cell involved. We consider the recognition repertoire, delivery of ligands to sensors, and spread of information to local and distal targets in the intact host. Finally, we consider the regulation of the innate immune response, appropriate to the challenge posed by infection.

Ligand diversity and receptor repertoire

Bacteria, fungi, and uni- and multicellular parasites express gene products that are foreign to the mammalian host, consisting of proteins (e.g. M proteins), carbohydrates (e.g. glucans), lipids (e.g. mycolates), or compounds of these molecules (e.g. LPS) (*Table 1*). These are therefore non-self ligands for opsonic and non-opsonic receptors of APCs. The response of the innate immune cells is influenced by whether such ligands are presented as a particle or as soluble molecules, in isolation or as part of an array of molecules present on an intact organism, able to engage multiple receptors at the same time. Whether the organism is living, able to secrete metabolites, invade and replicate, outside or within cells, or dead (depending also on the mode of death, e.g. heat treatment, complement-mediated lysis, antibiotic exposure, fixation) is also significant. As a result of antimicrobial defenses and digestion, extracellularly and intracellularly, ligands can be destroyed or progressively revealed, in a dynamic sequence, varying in stability and persistence. By contrast, viral glycoproteins are hybrids of foreign virus-encoded proteins and host-derived carbohydrates and lipids, perhaps favoring cross-reactivity and mimicry, with resultant autoimmunity. Nucleic acids present another complexity, e.g. the presence of CpG-like motifs on bacterial versus host DNA, DNA editing and modification, replicative intermediates in viruses, processed forms of RNA during replication, and virus transcription (64). These ligands would be mainly available within endosomes, the cytosol or other cellular compartments, including the endoplasmic reticulum, Golgi body, mitochondria, or even the nucleus, in a sea of host molecules of similar nature. Apoptosis and autophagy would also magnify the difficulties of a relatively simple self–non-self discrimination based on chemical structure. Finally, multicellular parasites are not only too large to ingest but can also mask their exposed surface molecules with host-derived components, thus avoiding recognition.

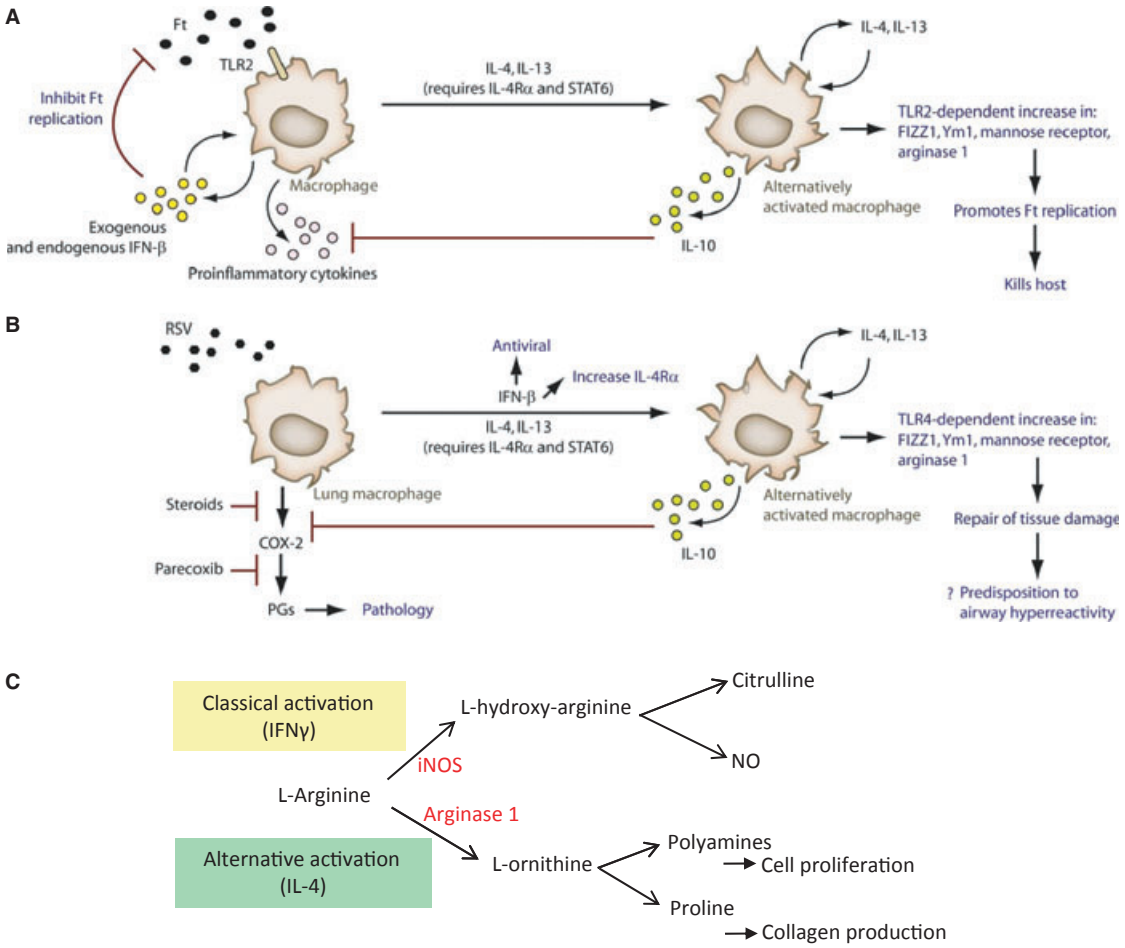

Fig. 4. Microbes induce macrophages to switch from a pro-inflammatory (M1) phenotype to an alternatively activated (M2) phenotype. (A) *Francisella tularensis* infection of macrophages results in an initial proinflammatory response through a TLR2-dependent pathway as well as an unknown intracellular sensor that leads to IFN-β production and activation of caspase-1 through AIM2 and ASC sensors. IFN-β acts to inhibit bacterial replication, but production of IL-4 and IL-13 differentiates the macrophages to an alternatively activated phenotype through an IL-4Rα- and TLR2-dependent mechanism that results in a microenvironment that promotes F. *tularensis* replication, which kills the host (62). (B) RSV infection of macrophages leads to an early induction of proinflammatory cytokines and COX-2, which has been shown to mediate the observed lung pathology. Macrophages then produce their own IL-4 and IL-13 and differentiate into alternatively activated macrophages through an IL-4Rα-, TLR4-, and IFN-β-dependent mechanism. This is thought to promote repair of damage caused by the earlier inflammatory process by inducing AAM markers including Arg1, FIZZ1, and Ym1. IL-10 acts to downregulate production of inflammatory cytokines (95). Reprinted from: Alternative activation of macrophages: mechanism and functions. Immunity 2010;**32:**593–604 (with permission from Elsevier). (C) IL-4 controls l-arginine metabolism in macrophages by modulating NOS/arginase 1 expression. Classically activated macrophages exhibit increased NOS activity, which promotes l-hydroxyarginine, l-citrulline, and NO production, contributing to their antimicrobial activity. By contrast, alternatively activated macrophages show increased arginase and decreased NOS activity. Here, l-arginine is metabolized to urea and l-ornithine which is metabolized to produce polyamines, molecules that induce cell proliferation, or proline, the basic building block of collagen, promoting tissue repair, and in pathological circumstances, excess fibrosis. Reprinted from: Alternative activation of macrophages: immune function and cellular biology. Immunobiology 2008;**214**:630–641 (with permission from Elsevier).

The germline-encoded receptor repertoire expressed by innate immune cells, is more diverse in terms of the range of ligands that can be recognized than the somatically rearranged receptors of B and T lymphocytes (65). There are no obvious holes in an innate repertoire that can react to proteins and peptides, carbohydrates, lipids and nucleic acids, including complex macromolecules such as glycolipids. In spite of the apparent promiscuity of scavenger receptors, for example, there is considerable specificity (66). Thus, although selected polyanionic and other ligands for SR-A I/II and MARCO overlap to a great extent, each receptor has distinct ligands, thus extending their potential combined repertoire. Receptors of all categories (opsonic, non-opsonic) can collaborate, yielding a multi-protein recognition complex, e.g., of CD14, MD2, and TLR4 to mediate sensing of LPS, and signaling (67). An analogous complex has been identified between MARCO and TLR2 in recognition of trehalose dimycolate, the cord factor of *M. tuberculosis* (68). Another example of combined

TLR-lectin signaling is the Dectin-1 mediated inflammatory response to β-glucan particles (69). Further distinct examples of receptor collaboration have been reported to involve CD36, mannose receptor (MR), and FcγRs (70). The key issue is therefore which receptor profile is expressed at a particular time and place by individual cells. In this regard, species differences, e.g. in TLR 7/9 expression, play an important role in determining immune function (71).

Returning to the question of self–non-self discrimination, it is important to recognize that the so-called pattern recognition receptors can and do react to altered self, such as acetylation or oxidation of low density lipoprotein, required to generate ligand for SR-A. Adhesion to a substrate may unfold protein or denature a non-ligand sufficiently to expose ligand potential. In the case of the mannose/fucose/GlcNAc receptor, selected glycoproteins such as lysosomal hydrolases, myeloperoxidase, salivary amylase, tissue plasminogen activator, and thyroglobulin acquire ligand-binding activity during normal biosynthesis but are cleared from an inappropriate extracellular compartment, such as the circulation, by macrophage endocytosis. The MR has a second lectin-like ligand-binding activity through its cysteine-rich domain, binding to sulfated-carbohydrate ligands in peripheral lymphoid organs, marginal metallophils in spleen, and subcapsular sinus macrophages in lymph nodes (72). This is responsible for non-immunogenic clearance of glycoconjugates, unless TLRs are co-incidentally stimulated, to induce a humoral response (73). One of the most confusing aspects of altered self-recognition relates to the nature of ligands and receptors involved in the clearance of cell debris, resulting from cellular injury or death, following infection or sterile injury. So-called 'danger' signals that have been proposed include the release of HMG-protein from chromatin, other alarmins (74), and undefined carbohydrate ligands for lectin-like receptors (75). Inflammasome activation has been ascribed to uric acid (76), and other crystalline materials. Oxidized lipids have also been implicated as inducers of innate immune responses following cell breakdown. The need for improved molecular characterization of ligands and receptors is obvious.

The role of myeloid cell-derived lysozyme in extracellular and intracellular breakdown of peptidoglycans, to generate NLR ligands such as muramyl dipeptide (MDP), has been neglected. Once ligand becomes available, e.g. following digestion and processing within endosomes, as also seen with delayed Dectin-1 recognition of *Aspergillus fumigatus* (77), there is the question of access to receptors in the appropriate cellular compartment. We have obtained evidence that the SR-A, for example, cannot activate NLR directly, but contributes to enhanced delivery of ligands such as MDP through endocytosis, while clearing potential ligand from access to surface-expressed TLR4 (S. Mukhopadhyay, unpublished observation). Delivery to endosomal TLR3, TLR7, or TLR9 would also be expected to enhance innate activation.

The SR-A also demonstrates another intriguing functional dichotomy. It is a phagocytic receptor for protein ligands on NM, mediating its uptake, while its LPS stimulates pro-inflammatory cytokine release (14). The MARCO SR, by contrast is absent on most tissue macrophages, but can be induced by TLR stimulation. Induction of MARCO is independent of the expression of SR-A but, once expressed, can enhance phagocytic uptake of these and other bacteria (23).

Regulation of innate immunity

How does the host regulate its innate immune response to infection? Starting again with surface molecules, the receptors described can synergize with one another, as described for Dectin-1 and TLR (27). Both receptors need to be signaling competent, e.g., through ITAM-like or TIR-domain motifs and TLR adapters such as MyD88, TIRAP/Mal, and Trif. Downstream signaling pathways for each receptor have been elucidated (Syk, Card9, malt, Bcl10) (78–80) and the NF-κB pathways (classical, alternative) (81), respectively. Other plasma membrane molecules are able to potentiate or downregulate innate reactivity. The EGF-TM7 family of serpentine myeloid surface molecules with large extracellular domains are structurally related to G protein-coupled receptors (although signaling has not been demonstrated). Ligation of one of these molecules, EMR2 (82), whose expression on monocytes, macrophages, and neutrophils is regulated by infection, potentiates myeloid cell responses to a range of agonists, including chemokine-induced migration, degranulation, and a respiratory burst. TREM-1 and TREM-2 on myeloid cells have very similar enhanced responses to stimulation (83, 84). By contrast, the CD200/CD200 receptor pair of IgSF molecules inhibits hyper-responses, induced by TLR, NLR, and inflammasome activation (34) (*Fig.* 5). In a *Neisseria* septicemia model in wildtype and CD200 knockout mice, bacterial loads were unaffected, but the CD200-deficient animals succumbed more readily to infection, since they were unable to limit TNF and IL-6 production at about 40 h postinfection. These experiments indicate that the balance between enhanced and reduced innate activation plays an important role in the host response to infection. It is likely that there will be a threshold to regulate potentially deleterious reactivity, which

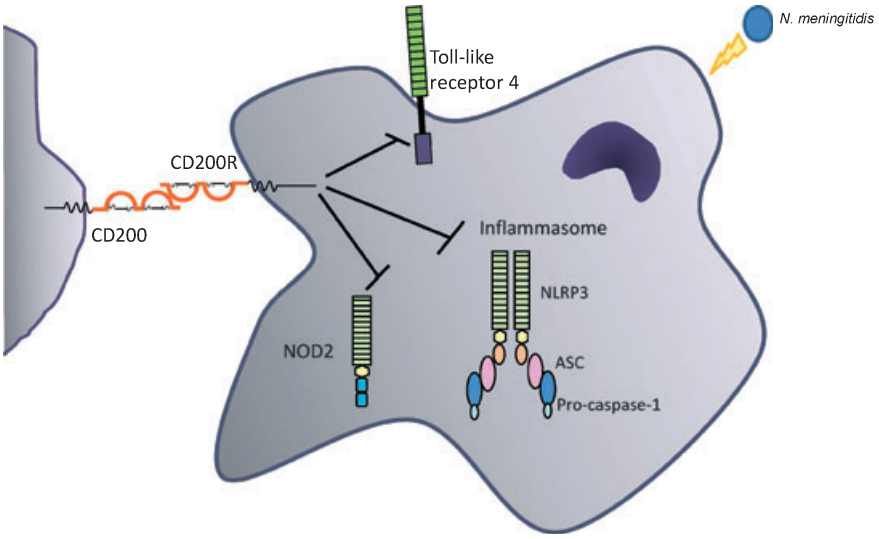

***Fig. 5.* CD200–CD200R interaction limits TLR and NLR function.** CD200 regulates the functions of three innate pathogen sensors: TLR4-mediated cytokine induction, NOD2 response and the inflammasome-mediated caspase-1 activation. Interaction of CD200 with CD200R from a cell exposed to a pathogen such as *Neisseria meningitidis* limits IL-1β secretion not just by inhibiting TLR-mediated pro-IL-β induction, but also by limiting inflammasome mediated caspase-1 activation. This inhibitory pathway plays an important role in controlling inflammation, thereby limiting processes such as bacterial sepsis (34) and excessive inflammation induced by viruses (96). ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; NLR, nucleotide-binding domain leucine-rich repeat containing family; NLRP3, NLR family pyrin domain containing 3; NOD2, nucleotide-binding oligomerization domain containing 2; TLR, Toll-like receptor.

may depend on intrinsic (e.g. genetic) factors as well as exogenous stimuli (e.g. infection).

Apart from the pro- and anti-inflammatory cytokines mentioned, type-1 interferon plays complex roles in protecting the host against viral and some bacterial infections, while exacerbating other bacterial infections, including experimental tuberculosis (85) and listeriosis (86). Interferon-γ, by contrast, primes the macrophage to evoke a potentiated response to LPS, promoting tissue injury and septic shock. Surprisingly, IL-4 pretreatment can also potentiate macrophage pro-inflammatory signaling and cytokine secretion after further challenge with TLRs utilizing particulate stimuli such as NM and zymosan (87). Since such combinations of Th2 environments, e.g. in acquired immunodeficiency syndrome and parasitic infections, are often found with TLR-dependent bacterial co-infections, it will be important to unravel the role of preconditioning by polarizing cytokines in cytokine shock syndromes. Finally, experimental models of tolerance to LPS, for example, indicate that there are powerful desensitizing mechanisms to dampen innate responses, some known to operate through modulation of the NF-κB pathway, which protect the host against hyperresponses and systemic injury (88).

Conclusion

We have taken a somewhat wider view of intracellular infection than usual, to consider the innate immune response to microorganisms of different classes and products derived extracellularly or intracellularly, which could act on the various recognition systems of phagocytic cells, especially macrophages. The ability of macrophages to distinguish among different classes of microbes can be established for fungi and some bacteria (Gram-negative and Gram-positive), but there is less knowledge of pathogen-specific recognition, which may be an exceptional event. In the case of *Neisseria*, a facultative intracellular Gram-negative diplococcus, macrophage SR-A does not distinguish among commensals, pathogens, laboratory strains, or even the closely related *Neisseria gonorrhoea* strain (12).

We have also not dealt with the parallel humoral system of innate immune recognition by the alternative complement activation pathway, mainly directed against extracellular pathogens, although complement receptor 3, for example, can contribute to entry of *M. tuberculosis* (89) and *Leishmania* into macrophages, even in the absence of exogenous complement. The presence of specific antibody or fixation of complement by the classical pathway has a major impact on the entry pathway, cellular responses, and outcome of infection, resulting in neutralization, or exceptionally, as in the case of Dengue Virus infection of macrophages, even enhanced infection (90).

We know little about the effects of established intracellular parasitism of macrophages on their ability to respond to repeated or different innate stimuli, whether tolerogenic or priming. The adaptive, more specific immune response also

can be expected to modulate macrophage innate responses further. The study of pathogen entry and innate immune responses has begun to probe a rich natural history, with diverse adaptations, providing fascinating insights into infectious disease but also illuminating cellular molecular mechanisms of pathogenesis.

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